



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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First-Named Inventor: George K. Stookey, PhD
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Examiner: Sayala, Chhaya D
Attorney Docket No.: 22076-3
Title: ORAL CARE CHEW PRODUCTS AND METHODS FOR DOMESTIC ANIMALS

DECLARATION OF DR. GEORGE K. STOOKEY UNDER 37 C.F.R. § 1.132

I, George K. Stookey, hereby aver as follows:

1. I am the George K. Stookey named as an inventor in the above-identified patent application.
2. I received a Bachelor's degree in Chemistry (1957) from Indiana University. I subsequently attended the Indiana University School of Dentistry, where I received a Masters degree in Preventive Dentistry (1962) and a Doctorate in Dental Sciences (1971).
3. In my work experience, in 1964, I joined the faculty in the Department of Preventive Dentistry at the Indiana University School Dentistry. I was promoted to Associate Professor at that institution in 1973, and to Professor in 1978. I served as the Associate Director of the Oral Health Research Institute from 1974-1981, and as Director from 1981-1999. I also served as the Associate Dean for Research at the Indiana University School of Dentistry from 1985-1996, and from 2000-2001. Beginning in 1996, I also served in administrative positions at

the Indiana University School of Dentistry including as Acting Dean (1996), Associate Dean for Academic and Student Affairs (1997-2000), and Executive Associate Dean (1998-2000). In 1998, I was promoted to Distinguished Professor at the Indiana University School of Dentistry. I continue to research actively with interests in fluoride pharmacology, the use of fluoride in preventing dental caries, in new technologies for the early detection of dental caries, and in technologies for the prevention of tartar buildup.

4. From the mid-1980's to the present, a significant focus in my research has been directed to the identification and development of systems for improving the dental health of companion animals such as dogs and cats. In this work, I was an inventor of and involved in the development of a hexametaphosphate-containing system for prevention of tartar buildup in animals. That system is now in use in several commercial products.

5. In my current patent application, I disclose the discovery of an animal chew product that improves the oral health of animals in respect of several measurable endpoints. The animal chew product incorporates both sodium tripolyphosphate and cetyl pyridinium chloride on or in an ingestible chew substrate.

6. Example 1 of my patent application sets forth *in vitro* zone of inhibition testing. In conducting Example 1, I discovered that cetyl pyridinium chloride (CPC) retains significant activity against bacterial organisms implicated in oral health issues, even in the presence of sodium tripolyphosphate (STP). In particular, a mixture including cetyl pyridinium chloride (0.4%) and sodium tripolyphosphate (0.72%) provided substantial zones of inhibition in respect of *Streptococcus mutans*, *Streptococcus sanguis*, and *Streptococcus parasanguis*.

7. Example 2 of my application summarizes a clinical dog study administered by trained and established clinical examiners. The study was a double blind, crossover study that

compared three regimens – (1) No Chew Product; (2) Chew Product with CPC-STP System; and (3) Chew Product Without CPC-STP System. Four-week test periods demonstrated that the inventive Chew Product with the CPC-STP system led to substantial reductions in both gingivitis and calculus, and also in dental plaque and mouth malodor.

8. I understand that my claimed invention has been denied a patent based upon an Office Action conclusion that as of the filing date of my application (July 18, 2003), it would have been obvious to one of ordinary skill in the art to carry out my invention with an expectation of success, in view of Spanier et al. (US Patents 5011679 and 5114704) in view of Witt et al. (US Patent 6350438) and further in view of Perlberg et al. (US Patent 6223643). Based upon my education and experience, I respectfully disagree with this conclusion.

9. My inventive chew product incorporates cetyl pyridinium chloride (CPC) in combination with sodium tripolyphosphate (STP). As of July 18, 2003, it would not have been possible to predict beforehand whether the incorporation of cetyl pyridinium chloride in the combination with STP in an animal chew product would provide a dental health benefit to animals. In this regard, I note that none of the patents cited in the rejection above contains any experiment conducted in animals testing a combination of STP and CPC for effects on oral health (in fact none of the patents contains a description of any experiment conducted in animals).

10. As evidence of this lack of predictability, the most extensively investigated and widely accepted antimicrobial agent for the prevention of oral disease is chlorhexidine (either the gluconate or acetate salt). As of the filing date of my patent application, hundreds of reports in the dental literature had documented the ability of topical rinses, solutions and gels containing this agent to reduce the formation of dental plaque, gingivitis and periodontal disease as well as

the development of dental caries in humans. Numerous reports in the dental literature also indicated that the application of these same products (particularly solutions and gels containing chlorhexidine) to experimental animals (typically rats and dogs) results in similar dental health benefits.

11. However, the incorporation of chlorhexidine into pet chew products, while having been attempted, did not result in dental health benefits. Rawlings et al., J. Vet. Dent. 15:129-134 (1998) (copy attached as Exhibit A), evaluated the impact of a dental hygiene chew product (Pedigree DentaboneTM) with or without 0.12% chlorhexidine included in the composition. After giving dogs a dental prophylaxis, they were provided once daily supplemental regimens involving either no treat, a chew product without chlorhexidine, or a similar chew product containing chlorhexidine. After three-week test periods in this three-week crossover study, the dogs were clinically examined for dental plaque, calculus and gingivitis. The authors reported that the “addition of chlorhexidine to the chew made no difference to the degree of gingivitis or the amount of calculus that accumulated....”.

12. Another reported investigation also evidences relative unpredictability in the incorporation of an antimicrobial agent into an animal chew product: Brown et al., J Vet Dent 22(1); 16-19 (2005) (copy attached as Exhibit B). In this investigation, designed as a 3-way crossover trial, the dogs were maintained on a basal diet consisting of a commercially-available premium dry diet mixed with a premium commercially-available canned diet in a ratio of 1:2 (a common practice in Australia). The three test regimens were: (a) no dental chew product; (b) a dental chew product; and (c) the same dental chew product to which 0.2% of a natural antimicrobial agent had been added. The identity of the antimicrobial agent was not disclosed and was noted as an “undisclosed, proprietary product”. When indicated by test design, a chew

product was provided to each dog one hour prior to feeding the basal diet. At the beginning of each 6-week experimental period the dogs had their teeth cleaned and polished to remove all exogenous deposits (dental prophylaxis) and their teeth were brushed once daily with a dentifrice for 2 weeks to obtain optimal gingival health. The dogs were then anesthetized, examined for gingivitis, given another dental prophylaxis and provided the test regimen without further toothbrushing for 4 weeks with clinical examinations for plaque, calculus and gingivitis performed at the end of the 4-week test period. The results of the study indicated that while the supplemental provision of the chew product resulted in significant reductions in the accumulation of plaque and calculus as well as the development of gingivitis, "the inclusion of the antimicrobial agent did not improve the efficacy of the product".

13. In light of these factors, prior to my invention, the efficacy of the animal chew product containing CPC and STS in significantly reducing both gingivitis and dental calculus, as demonstrated in my patent application, could not have been predicted.

14. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on that information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by a fine or imprisonment, or both, under §1001 of Title XIX of the United States Code and that such willful false statements may jeopardize the validity of the application and/or patent issued therefrom.

Date: November 26, 2007

George K. Stookey
George K. Stookey, PhD

Effect on Canine Oral Health of Adding Chlorhexidine to a Dental Hygiene Chew

J.M. Rawlings, PhD
C. Gorrel, Vet MB, DDS
P.J. Markwell, BVetMed



Summary: A study to compare the effect of a dental hygiene chew with or without 0.2% chlorhexidine on the development of gingivitis and the accumulation of dental deposits was performed using 11 small dogs. Confirming previous data, the daily addition of a standard chew to a dry diet resulted in significantly less gingivitis and calculus after 3 weeks compared with feeding the dry diet alone. Addition of chlorhexidine to the chew made no difference to the degree of gingivitis or the amount of calculus that accumulated, but did result in significantly less plaque accumulation after 3 weeks. The abrasive-ness of the chew, rather than the antibacterial activity of chlorhexidine, is likely to have contributed the most to the maintenance of oral health in dogs with mild gingivitis.

J Vet Dent 15(3); 129-134, 1998.

Introduction

Supragingival plaque is generally recognized to be the primary etiological factor for gingivitis in dogs.¹⁻³ Although gingivitis does not inevitably progress to periodontitis,⁴ it may be an important predisposing factor for disease progression.⁵ Hence, a logical approach to the prevention of periodontal disease is meticulous supragingival plaque control.

Several studies have shown that scrupulous mechanical hygiene procedures can successfully prevent periodontal disease in dogs.⁶⁻¹⁰ However, the level of dedication and motivation required to achieve and maintain periodontal health in dogs is not readily sustained by most owners.¹¹ More typically there is a temporary benefit to gingival health in response to periodontal therapy, tooth brushing instruction, and initial enthusiasm. Because many owners may not brush their dog's teeth as frequently as required to maintain periodontal health, a range of chewing devices designed to remove supragingival plaque is now available. In a previous study, we reported that the daily addition of an appropriately designed dietary chew to a regimen of tooth brushing every other day was better than tooth brushing alone in helping to maintain oral hygiene in dogs.¹²

Incorporation of chemical antiplaque agents in oral hygiene products is a way of augmenting mechanical cleaning procedures in controlling supragingival plaque formation, thus helping to prevent the onset of early periodontal disease.

Chlorhexidine (1,1'-hexamethylene bis(5-(p-chlorophenyl) biguanide) is the most effective and most thoroughly tested chemical antiplaque and antigingivitis agent.¹³⁻¹⁶ Chlorhexidine is a broad spectrum antimicrobial with substantial residual activity that has very low toxicity. It is effective in inhibiting plaque accumulation on tooth surfaces in dogs,^{16,17} altering the composition of the microbial flora and decreasing the quantity of bacteria in saliva.^{15,18} It exerts its bacteriostatic and bacteriocidal actions by disrupting the lipoproteins in bacterial cell walls and then penetrating the cells where it causes precipitation of the cytoplasm.¹⁵

The purpose of this study was to assess if incorporation of chlorhexidine digluconate into a dental hygiene chew, previously shown to have efficacy in reducing plaque accumulation and gingivitis,¹⁹ would further improve upon the periodontal health achieved by daily feeding of the dental hygiene chew.

Materials and Methods

The study was conducted at the Waltham Centre for Pet Nutrition, Waltham-on-the-Wolds, Leicestershire, United Kingdom. The colony consisted of 11 small dogs (weight range 6.5-10.4 kg; median 7.3 kg). Details of breed, age, and sex are summarized in Table 1. The animals were housed in pairs in purpose-built kennel facilities²⁰ with raised heated flooring, free access to an external run, and daily exercise in a grassed paddock area. Each dog was also lead-walked outdoors for at least 10 minutes

Table 1. Breed, age, sex, and weight of dogs used in the study.

Dog	Breed	Age	Sex	Weight
P9	Miniature poodle	3	MC	9.5
P10	Miniature poodle	3	MC	10.4
C103	Cairn terrier	5	MC	7.7
C104	Cairn terrier	5	MC	8.5
C110	Cairn terrier	2.5	FS	7.3
C111	Cairn terrier	1.5	MC	6.5
C118	Cairn terrier	1.5	FS	6.8
LHD1	Long-haired dashchund	2.5	FS	7.3
LHD3	Long-haired dashchund	2	MC	5.3
Y1	Yorkshire terrier	8	MC	7.3
W110	West Highland white	6	FS	7.5

• Age in years; weight in kilograms.
• MC = Male, castrated; FS = Female, spayed.

per day. Throughout the study the dogs were fed once daily and water was provided *ad libitum*. The dogs remained healthy throughout the study, as determined by clinical examination and blood biochemistry screens prior to the start of and on completion of the trial.

Study Design

The study was conducted in a Latin square design, where each study period consisted of a 1-week pretest phase, followed by a 3-week test phase. The 1-week pretest phase consisted of daily toothbrushing with a toothpaste marketed for use in dogs^a followed by dental scaling and polishing. The dogs were scored for gingivitis prior to the dental scaling and polishing and at the end of the pretest phase to obtain a baseline score for gingival inflammation prior to the start of the test phase. At the end of the pretest phase, the teeth were again scaled and polished to ensure a clean tooth surface, supra-and subgingival, at the start of the test phase.

All dogs were fed a nutritionally complete canned diet^b during the pretest phase, and were then randomly assigned to one of the dietary test regimens for a period of 3 weeks. These regimens consisted of:

- 1) a nutritionally complete premium dry diet^c fed as the control diet,
- 2) a dry diet fed in combination with a dental hygiene chew^d given once daily, and
- 3) a dry diet fed in combination with the dental hygiene chew incorporating chlorhexidine gluconate at a concentration of 0.2% w/w.

Food allowances were based on maintenance energy requirements, calculated as $110 \times \text{body weight (kg)}^{0.75}$ kcal/dog/day. Body weight was monitored weekly and food allowances adjusted accordingly. The amount of complete dry diet was reduced to take into account the energy content of the dental hygiene chew.

Gingivitis and plaque were scored after 1 week; calculus, stain, gingivitis, and plaque were scored after 2 additional weeks (the indices were always scored in this sequence). Following an additional dental scale and polish procedure, the dogs were crossed into the next period of study, commencing with the pretest phase.

The examinations and dental scaling and polishing were performed under general anesthesia. The animals were premedicated with acepromazine^e (0.2mg/kg) and atropine sulphate^f (60 micrograms/kg) by subcutaneous injection. Anesthesia was induced with propofol^g (4mg/kg IV) and maintained on oxygen/halothane^h via a cuffed endotracheal tube. Dental scaling and polishing were performed using a dental unit fitted with a sonic scalerⁱ, and the gingival sulci were irrigated with deionized water.

^a Dentipet® toothpaste, Arnold's Veterinary Products, Shrewsbury, United Kingdom.

^b Pedigree® Chum®, Pedigree Petfoods, Melton Mowbray, United Kingdom.

^c Pedigree® Veterinary Plan 360/29®, Pedigree Petfoods, Melton Mowbray, United Kingdom.

^d Pedigree® Rask®/Dentabone®, Thomas's, Birstall, United Kingdom. Note: Marketed as Pedigree® Chum® Rask® in Europe and as Pedigree® Dentabone® in North America.

Oral Assessment Methods

The methods used for scoring gingivitis and dental deposits in this study were human techniques modified for veterinary use, and are summarized in Tables 2 through 5. In addition to measuring the standard indices of oral hygiene, samples of plaque were removed from the gingival margin of the left maxillary fourth premolar (208) and the left mandibular fourth premolar (308) teeth before and after all three trial periods. These samples were submitted for bacterial culture. Details of the methods used and results obtained will be presented in a separate paper. However, since plaque was sampled from the left half of the mouth, deposits of plaque were measured only on the right mandible and maxillary teeth.

Data and Statistical Analysis

The values for each parameter measured, except for plaque, are expressed as whole mouth scores. This refers to the mean value of the scores obtained for each tooth examined, as described in Tables 2 through 5. Since plaque was sampled from selected teeth on the left half of the mouth for a separate study, plaque is expressed as the average score for the combination of right maxillary and right mandibular teeth. Means and standard deviations are used to summarize these scores.

Analysis of variance methods were used to derive F-tests for significant differences between sequences, significant differences between periods, and significant differences between treatments. F-values with p-values less than 0.05 were considered significant. Analyses were performed using ANOVA/GLM procedures in Minitab V9 statistical software.

RESULTS

Gingivitis

After 1 week of daily tooth brushing during the pretest phase, gingivitis for individually scored teeth was rated either 0 or 1 according to the scoring definition described in Table 2, providing an average whole mouth score of approximately 0.5 (Fig. 1). Subjectively, the gingivae were regarded as clinically healthy and there was no statistical difference in the mean baseline gingivitis scores between groups. Feeding a dry diet alone, without tooth brushing, resulted in a progressive increase in gingivitis during the 3-week test phase. However, when the dental hygiene chew was fed with the dry diet, there was significantly less gingivitis both after 1 week (34%; $p < 0.002$) and 3 weeks (25%; $p < 0.001$) compared with the dry diet alone. This was also true when the chlorhexidine chew was fed with the dry diet, resulting in 31% and 35% less gingivitis after 1 and 3 weeks, respectively. When data for the standard and chlorhexidine chew were compared, there was no significant difference in gingivitis after 1 or 3 weeks.

^e C-Vet, Leyland, United Kingdom.

^f C-Vet, Leyland, United Kingdom.

^g Mallinckrodt, Uxbridge, United Kingdom.

^h Rhone Merieux, Harlow, United Kingdom.

ⁱ Cenvet iM3, Veterinary Instrumentation, Sheffield, United Kingdom.

Table 2. Gingivitis

Score	Category	Description
1	Mild inflammation	Slight redness and swelling but no bleeding, or delayed bleeding on gentle probing of the gingival sulcus.
2	Moderate inflammation	Gingiva is red, swollen, and bleeds on gentle probing of the sulcus.
3	Severe inflammation	Gingiva is red or reddish-blue, the gingival margin is swollen, tendency to spontaneous hemorrhage or profuse hemorrhage on probing, and ulcerations along the gingival margin.

- A modified Loe & Silness gingival index (GI) was used. The modification involved measuring gingivitis along only the buccal gingival sulcus and only recording one value per tooth.
- Teeth scored: Maxilla I3, C, P2, P3, P4, M1; Mandible C, P2, P3, P4, M1.
- Calculations: Mean mouth gingivitis score is mean of all teeth scored.

Table 3. Plaque

Score	Coverage	Score	Thickness
0	No observable plaque	0	No observable plaque
1	1-24% coverage	1	Light = pink to light red
2	25-49% coverage	2	Medium = red
3	50-74% coverage	3	Heavy = dark red
4	75-100% coverage		

- Modification of Quigley and Hein (Turesky) index. Plaque was disclosed by applying the disclosing solution to the buccal surface of each tooth and immediately rinsing with water. The gingival and coronal halves for each tooth were scored separately.
- Teeth scored: Right maxilla I3, C, P2, P3, P4, M1; Right mandible C, P2, P3, P4, M1
- Disclosing solution: tablets crushed and dissolved in deionized water to give a concentration of 2 mg/ml erythrosine.
- Calculations: The score for each tooth half was calculated by multiplying the coverage and thickness scores. Gingival and coronal scores were then added together to give the tooth score. The sum of the teeth scores was termed the total tooth score. The score for the whole mouth was the mean of all teeth scored.

Dental Deposits

All dogs had their teeth scaled and polished, so no dental deposits were present at the start of the test phase (calculus, plaque, and stain indices were 0 in all dogs). For all dietary regimens, calculus accumulated during the 3 weeks of feeding (Fig. 2), but the amount of calculus was not extensive. There was, however, a significant ($p < 0.001$) difference between regimens. Compared with the dry diet alone, the addition of the standard chew resulted in 54% less calculus accumulation, while feeding the chlorhexidine chew produced 66% less calculus accumulation. There was no statistical difference in calculus accumulation between the standard and chlorhexidine chews.

Plaque accumulation on the right half of the mouth was measured after both 1 and 3 weeks of feeding the diets (Fig. 3). At both time periods, the addition of the standard chew to a dry diet did not result in any difference in plaque accumulation compared with the dry diet alone. In contrast, feeding the chlorhexidine chew produced significantly less plaque accumulation after 1 week ($p < 0.024$) and after 3 weeks ($p < 0.018$) compared with both the dry diet alone and the standard chew. The chlorhexidine chew resulted in 17% and 27% less plaque accumulation after 1 and 3 weeks, respectively, compared with the standard chew.

In this study, stain was measured after 3 weeks of

feeding each dietary regimen (Fig. 4). The whole mouth score for stain after feeding the dry diet was 2.16 ± 1.15 ; after feeding the standard chew it was 1.64 ± 0.88 . When the chlorhexidine chew was fed, the whole mouth score for stain was 1.61 ± 0.86 . There were no significant differences between any of the dietary regimens.

Table 4. Calculus

Score	Coverage
0	No observable calculus
1	1-24% coverage
2	25-49% coverage
3	50-74% coverage
4	75-100% coverage

- Modified Schiff method. The teeth were gently air dried to increase visualization. The buccal surface of the tooth was divided vertically into mesial, buccal and distal thirds, and each third was assigned a numerical score based on coverage.
- Teeth scored: Maxilla: I3, C, P2, P3, P4, M1; Mandible: C, P2, P3, P4, M1.
- Calculations: The tooth score is the sum of the scores for each of the three tooth surfaces. The sum of the teeth scores is averaged to obtain a whole mouth mean calculus score.

Table 5. Stain

Score	Coverage	Score	Intensity
0	No observable stain	0	No observable stain
1	1-24% coverage	1	Light yellow or tan
2	25-49% coverage	2	Medium brown
3	50-74% coverage	3	Dark brown to black
4	75 - 100% coverage		

• Modified Schemehorn method. The buccal surface of the tooth was divided vertically into mesial, buccal and distal thirds, and each third was assigned a numerical score based on coverage and an intensity score.
 • Teeth scored: Maxilla I3, C, P2, P3, P4, M1; Mandible C, P2, P3, P4, M1.
 • Calculations: The score for each tooth site (mesial, buccal, distal) was calculated by multiplying the coverage and intensity scores. The resultant three scores were summed to obtain a tooth score. The teeth scores (total tooth scores) were averaged to calculate the whole mouth mean stain score.

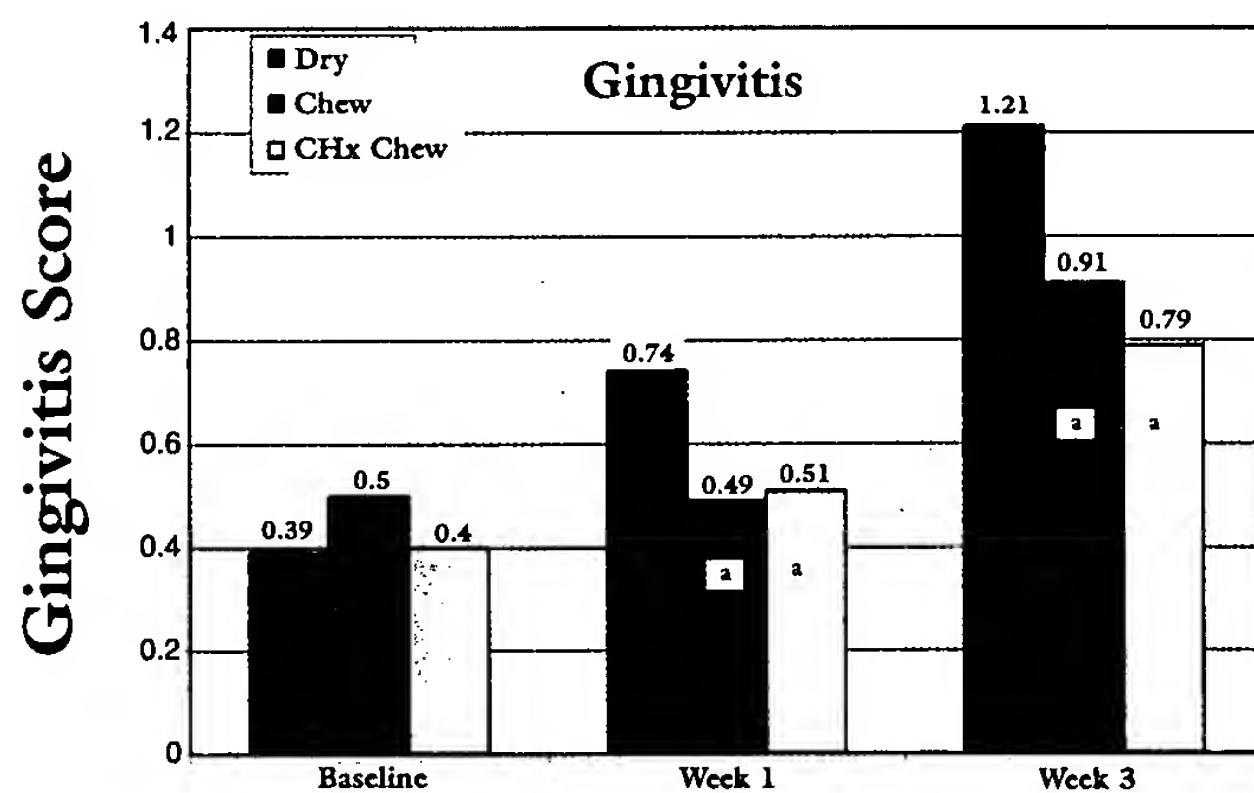


Figure 1. Effect of a dental hygiene chew with and without 0.2% chlorhexidine on the mean whole mouth gingivitis score. Gingivitis was measured after 1 week of tooth brushing (Baseline) and after 1 and 3 weeks of feeding each dietary regimen. a=significantly different compared with a dry diet alone.

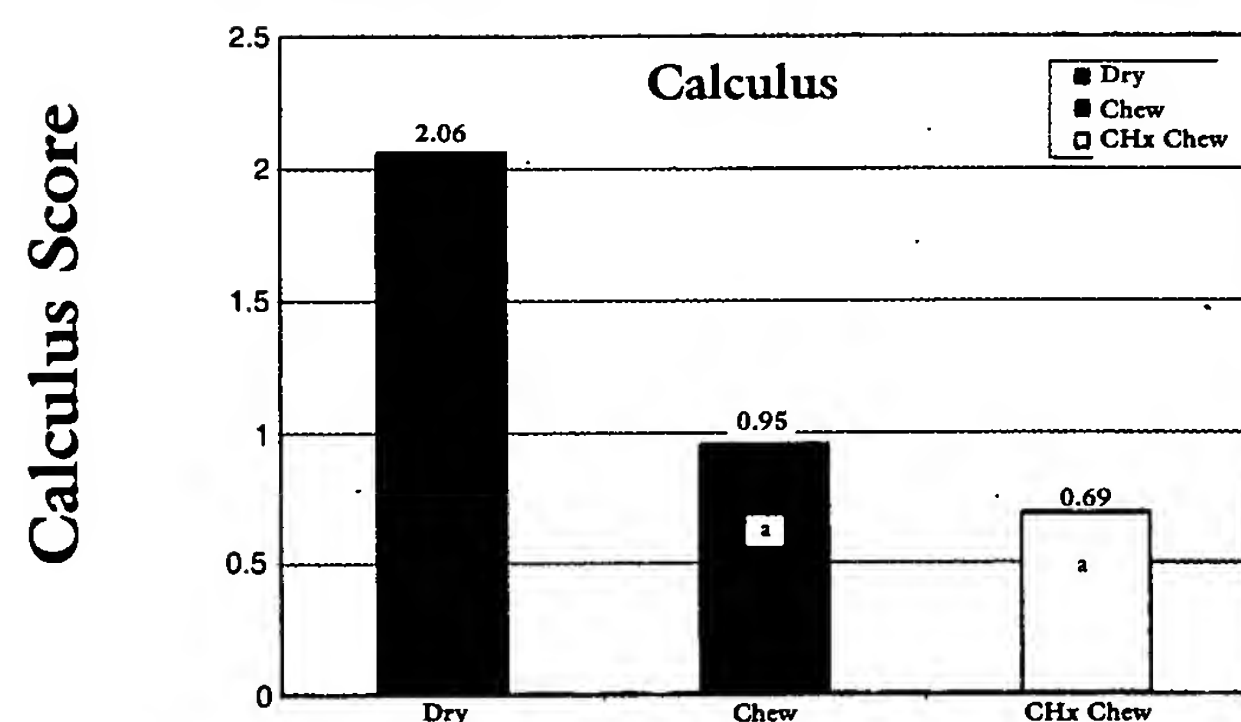


Figure 2. Effect of a dental hygiene chew with and without 0.2% chlorhexidine on the whole mouth mean calculus score after 3 weeks of feeding each dietary regimen. a=significantly different compared with a dry diet alone. For all dogs, the calculus index for all teeth was 0 at the start of the trial period.

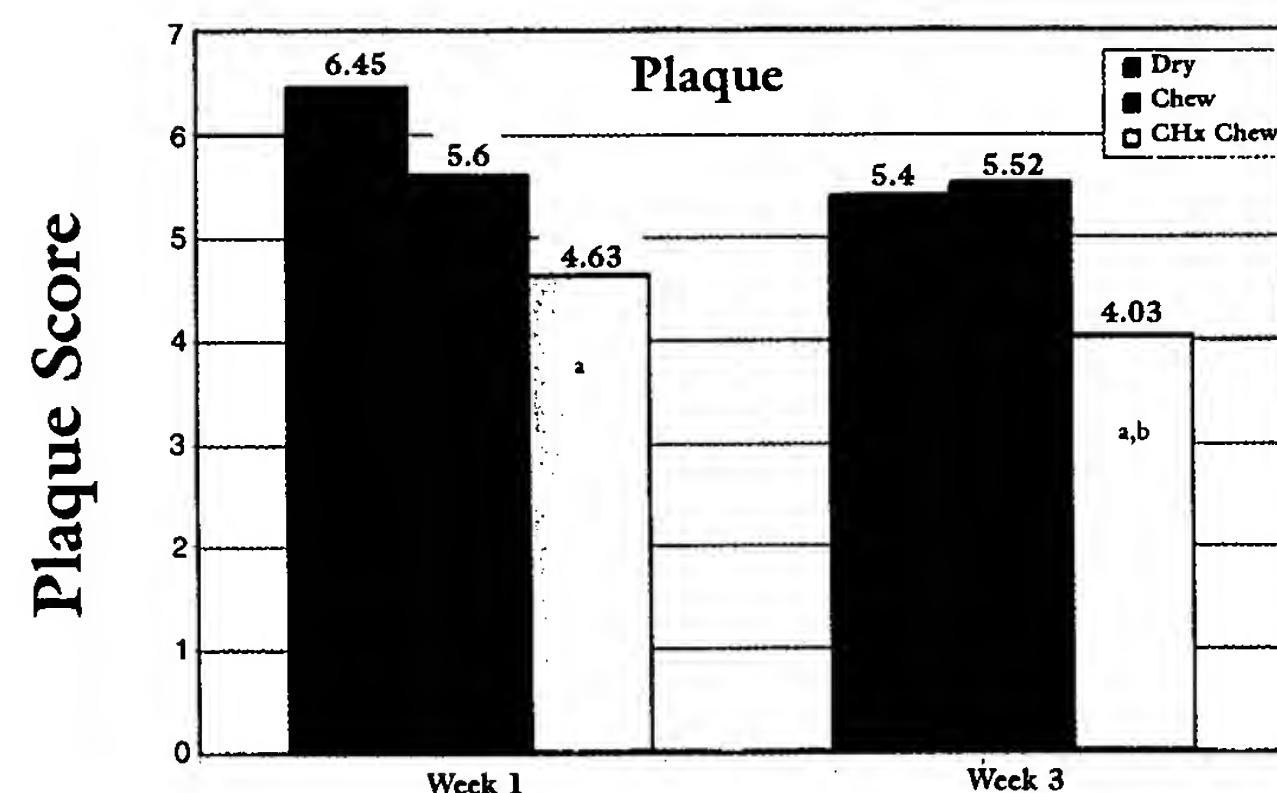


Figure 3. Effect of a dental hygiene chew with and without 0.2% chlorhexidine on the mean mouth plaque score (right side only). Plaque was measured after 1 and 3 weeks of feeding each dietary regimen. a=significantly different compared with a dry diet alone; b=significantly different compared with the standard chew. For all dogs, the plaque index for all teeth was 0 at the start of the trial period.

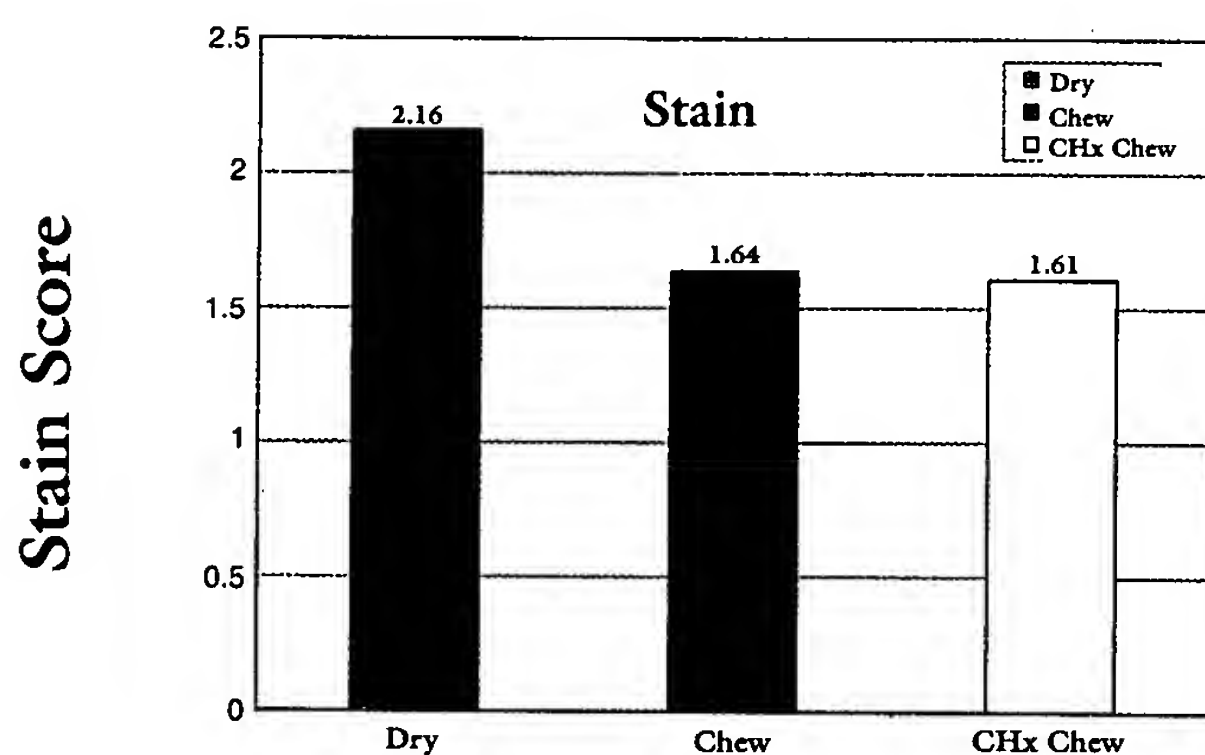


Figure 4. Effect of a dental hygiene chew with and without 0.2% chlorhexidine on the whole mouth mean stain score after 3 weeks of feeding each dietary regimen. For all dogs, the stain index for all teeth was 0 at the start of the trial period.

DISCUSSION

In a previous study, we demonstrated that the daily addition of an appropriately designed chew to a dry diet main meal is effective in reducing accumulation of dental deposits on teeth, as well as reducing the severity of gingivitis compared to the regimen of dry diet alone.¹⁹ The presently reported study also showed a significant reduction in the development of gingivitis and in the accumulation of calculus after 3 weeks. There was significantly less plaque accumulation after 1 week in the first study, but in both studies by 3 weeks there was no difference whether the dry diet was fed with or without the chew. Hence, the reproducibility of our findings has been achieved using identical protocols in small dogs of different breeds, in two different locations and with two commercially available dry diets, all of which are important criteria for validation of any oral hygiene product.

In the current study, the incorporation of 0.2% chlorhexidine in the dental hygiene chew resulted in significantly less plaque accumulation after 3 weeks compared with both the dry diet alone and the standard chew. Despite this reduction in plaque accumulation, there was no appreciable benefit of adding chlorhexidine to the chew with regard to calculus accumulation and the development of gingivitis. In addition, there was no significant difference in tooth staining between either chew despite the fact that a significant side effect of chlorhexidine is its propensity to stain the tooth enamel.¹⁵ It seems likely that the mechanical abrasion of the dental hygiene chew minimized the staining that might otherwise have resulted from use of chlorhexidine. Other reported side effects of chlorhexidine, such as reduced palatability and mucosal erosion,¹³ were not observed in this study.

There does not seem to be a simple relationship between the amount of plaque on the tooth surface and the severity of gingivitis that develops. When dogs were fed the standard chew in addition to their dry diet, there was the same amount of plaque after 3 weeks as when they received a dry diet alone. There was, however, significantly less gingivitis. In contrast, the addition of chlorhexidine to the chew reduced the total amount of plaque but was not effective at reducing gingivitis compared to the standard chew.

Plaque accumulation was determined in this study by measuring the percentage of tooth covered and the intensity of staining by the disclosing solution¹; the stain intensity was used as an indicator of plaque

thickness. It is plausible that it is the composition of plaque, rather than the total amount of plaque, which is an important factor in the development of gingivitis. Through the abrasive action of the chew, most existing plaque will be removed but new plaque will be deposited very rapidly between meals. Consequently, although the area of tooth covered by plaque may remain relatively constant, the composition of the plaque never has the opportunity to evolve and mature into a state that favors the proliferation of bacterial populations associated with the development of periodontal disease. Although chlorhexidine reduced the total amount of plaque coverage, which is in keeping with chlorhexidine's potent antibacterial properties,¹³⁻¹⁶ it is possible that it had little additional effect on the plaque bacteria situated subgingivally or at the gingival margin.

A number of factors may account for chlorhexidine's lack of effect on gingivitis. The concentration of chlorhexidine used in this study (0.2% w/w) is typical of that incorporated into many existing proprietary products, but the effective concentration may be somewhat less if chlorhexidine were to bind to organic materials as has been reported.¹⁵ Unpublished *in vitro* microbiology studies in our laboratory confirm that chlorhexidine is released in an active form from the chew, but whether this is sufficient to affect plaque bacteria sited subgingivally has not been established. Alternatively, chewing devices may not be the most effective means of promoting penetration of chlorhexidine into the gingival sulcus, since delivery to, and contact time with affected areas may be less than optimal.

The effectiveness of the chew is thought to be due partly to its abrasive action. It is also possible that the physical act of chewing may influence the development of periodontal disease, perhaps through altering the bacterial population or changes in host response to infection. Chewing in humans promotes salivary secretions, which have a direct effect on periodontal health through changes in oral pH, the secretion of IgA, lysozyme, lactoferrin and lactoperoxidase,²¹⁻²³ and which flush the bacteria from the surface of oral tissues.²¹ Therefore, good balanced nutrition combined with chewing activity will confer improved health status and a greater ability to respond to infection.

¹ Disclosing Tablets, Boots, Nottingham, England. Note: tablets crushed and dissolved in deionized water to achieve a solution of 2 mg/ml erthrosine.

This study, together with several others,^{6-10,19,24} has shown that clinically healthy gingivae can be achieved by daily tooth brushing. Once tooth brushing stops, gingivitis starts to develop irrespective of the diet fed.^{12,19,24} The type of main meal diet which is fed may be less important to the subsequent development of periodontal disease than the use of adjunctive therapy such as that obtained using dental hygiene chews or similar materials.

We conclude that the daily inclusion of a dental hygiene chew to a dry food regimen reduced the degree of gingivitis and the level of calculus accumulation when compared with the dry diet alone in small dogs. The incorporation of chlorhexidine in the chew resulted in a reduction in plaque accumulation, but there was no additional benefit to the mechanical effects of the chew on gingival health, or on the development of calculus deposits.

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Author Information

From the Waltham Centre for Pet Nutrition, Waltham-on-the Wolds, Melton Mowbray, Leicestershire, LE14 4RT, United Kingdom (Rawlings and Markwell) and 17 Burnt House Lane, Pilley, Near Lymington, Hampshire, SO41 5QN, United Kingdom (Gorrel).

Effective Periodontal Disease Control Using Dental Hygiene Chews

Wendy Y. Brown, MscAg; Phil McGenity, BA, PhD, C. Chem

Summary:

*This study evaluated the effectiveness of a newly developed dental hygiene chew for dogs, with and without a natural antimicrobial additive, compared with a reference diet. Efficacy was determined by measuring the severity of gingivitis and the accumulation of dental plaque and calculus in dogs after 4-weeks of being fed the different dietary regimens. Dogs fed a single daily dental chew had significantly less gingivitis ($P = 0.02$), plaque ($P = 0.0004$), and calculus ($P = 0.0001$) compared with dogs in the control group that were fed an identical diet but received no chews. The inclusion of the antimicrobial agent did not improve the efficacy of the product. The dental hygiene chews tested in this study have potential to help reduce the incidence of periodontal disease in dogs. *J Vet Dent* 22(1); 15-19, 2005*

Introduction

Periodontal disease has been identified as the most frequently occurring clinical condition in domestic dogs and cats.¹ The disease begins with the accumulation of bacterial plaque on the tooth surfaces initiating an inflammatory response that affects the supporting tissues of the tooth and eventuates in loss of attachment.² The texture of the habitual diet can be a major contributing factor, with gingivitis and dental deposits being most extensive when dogs are fed soft diets.³⁻⁵ Interestingly, no such relationship has been established with dietary components.⁶ Periodontal disease occurs most frequently in small breeds of dogs, and in all dogs the incidence of periodontal disease increases with advancing age.⁷ As the disease progresses, feeding becomes difficult and painful, and tooth extraction is often recommended, which can be both traumatic and hazardous, particularly for the elderly dog.

Current recommendations for reducing the incidence of periodontal disease in dogs include the feeding of hard rather than soft diets, and the provision of chewing material such as bones or rawhide strips.⁸ However, hard kibbled diets do not necessarily provide the most optimum texture, and on their own they do not prevent the accumulation of dental deposits in dogs. Tooth brushing has been found to be the most effective method of preventing periodontal disease in dogs,⁹ but is only effective when brushing is performed at least 3 times a week,¹⁰ and many pet owners are not willing to comply with this regimen.¹¹ Control of dental deposit accumulation in dogs by dietary means offers a more realistic approach, and much of the research in recent years has focused on the development of specially textured diets or dietary supplements (dental chews, rusks, or rawhide strips). These products aim to reduce the incidence of periodontal disease in dogs either by preventing or removing the build-up of dental deposits. The feeding of a dietary supplement, such as a dental chew, rather than a specialized diet has the advantage that the dog's usual diet

can be fed and varied at will, without restriction. The dental chew, being a highly palatable treat, is well accepted and can be offered in addition to the normal diet at any convenient time. In studies designed to test the effectiveness of dental hygiene chews in dogs, a significant reduction in the accumulation of dental deposits was reported when the diet was supplemented daily with a single dental hygiene chew.¹²⁻¹⁵

The objective of this study was to evaluate the effectiveness of two newly developed dental hygiene chews, relative to a reference diet. One of the chews evaluated in this study (Chew A) has recently become commercially available in Europe^b. The second chew (Chew G) was identical to the first except that it contained a natural antimicrobial additive*. Efficacy was determined by measuring the severity of gingivitis and the accumulation of dental plaque and calculus (clinical signs of periodontal disease) in dogs fed the different dietary regimens.

Materials and Methods

The study was conducted at the University of New England (UNE) dog holding facilities at Armidale, NSW (Australia). Twelve dogs of mixed breeds participated in the study consisting of 7 males (2 neutered) and 5 females (1 neutered). The bodyweights of the dogs measured at the beginning of the study ranged from 3 to 15.2 kg, and ages ranged from 1 to 10-years. Authority to conduct this study was granted by the UNE Animal Ethics Committee (AEC) in accordance with Section 25 of the Animal Research Act (1985). Animals received the highest standard of care throughout the study in accordance with UNE AEC guidelines, and in compliance with Waltham Ethical Guidelines.

The study was conducted as a 3 x 3 Latin Square, with the following 3 dietary regimens fed over 3 periods: (1) Standard Diet + Chew A; (2) Standard Diet + Chew G; (3) Standard Diet + No Chew (Control). The standard diet was a premium complete and balanced commercial dry dog food^c, fed in combination with a

Figure 1

Photograph showing the size and shape of the dental chews tested in this study.



Table 1: Gingivitis Scoring Criteria

Gingivitis was assessed using a modification of the method designed by L  e & Silness¹⁶. The buccal gingiva for each tooth was visually divided into thirds (mesial, buccal, and distal). Each site was evaluated by the criteria below and given a score between 0 and 4.

0	No gingivitis	
1	Slight inflammation	slight redness but no bleeding on probing
2	Mild inflammation	slight redness and swelling, with delayed bleeding on gentle probing of the gingival sulcus
3	Moderate inflammation	the gingiva is red, swollen and bleeds on gentle probing of the sulcus
4	Severe inflammation	the gingiva is red or reddish-blue, the gingival margin is swollen, tendency to spontaneous hemorrhage or profuse hemorrhage on probing and/or ulcerations along the gingival margin

Calculations: A total tooth score for each tooth was obtained by adding together the scores from each of the three sites. The tooth scores from all teeth scored were then averaged to obtain a mean whole mouth score for each dog.

Table 2: Plaque Scoring Criteria

Plaque was scored using the Quigley and Hein method¹⁷ as modified by Turesky¹⁸. Plaque was disclosed by applying dental disclosing solution¹ undiluted to the buccal surface of each tooth and immediately rinsing with water. The gingival and occlusal half for each tooth were scored for plaque coverage and plaque thickness, using the following scoring system:

Coverage		Thickness	
0	no observable plaque	1	Light = pink to light red
1	1 - 24 % coverage	2	Medium = red
2	25 - 49 % coverage	3	Heavy = dark red
3	50 - 74 % coverage		
4	75 - 100 % coverage		

Calculations: The "cover" score was multiplied by the "thickness" score for both the gingival and occlusal portions of each tooth. The gingival and occlusal values for each tooth were then added together to obtain a total tooth score. The total tooth scores from all teeth were then averaged to obtain a mean whole mouth score for plaque.

Table 3: Calculus Scoring Criteria

Calculus was assessed using the method of Warrick and Gorrel¹⁹. The disclosed plaque was first removed with a toothbrush and then rinsed from the teeth using a dental air-water syringe. Teeth were then air-dried. The buccal surface of each tooth was visually divided vertically into mesial, buccal, and distal thirds and each third was assigned a numerical score for both coverage and thickness. A probe was used gently to verify the visual impression of coverage and thickness.

Coverage		Thickness	
0	no observable plaque	1	Light < 0.5 mm
1	1 - 24 % coverage	2	Medium 0.5 - 1.0 mm
2	25 - 49 % coverage	3	Heavy > 1.0 mm
3	50 - 74 % coverage		
4	75 - 100 % coverage		

Calculations: The coverage score was multiplied by the thickness score for each tooth surface. The total tooth score is the sum of the scores for each of the three tooth surfaces. The total tooth scores from all teeth were then averaged to obtain a mean whole mouth score (for calculus) for each animal.

premium complete and balanced commercial tinned dog food^d, in a ratio of 1:2 by weight. Animals were fed maintenance energy requirements (MER) as determined by the formula $MER (kcal) = 140 \times BW(kg)^{0.75}$ once daily in the afternoon, and any refusals were weighed and recorded. Bodyweights were measured at 2-week intervals, and amounts fed were adjusted as necessary to maintain ideal bodyweights. The products tested in this study were two variations (a standard and a modified form) of a dental hygiene

chew for dogs^e (Fig. 1). **Chew A: Standard Chew-** An extruded starch-based dental chew, containing no antimicrobial additives. Its activity is based solely on its texture. **Chew G: Modified Chew** - Contains 0.2 % of a natural antimicrobial agent.

Each period of the experiment consisted of a 2-week pre-test phase followed by a 4-week test phase. On day 1 of the pre-test phase all dogs had their teeth scaled and polished under general anesthesia. Dogs' teeth were then brushed once daily in the

Table 4: Results of Gingivitis, Plaque, and Calculus Scoring

Group means (\pm SEM) for whole mouth scores for gingivitis measured before and after treatments.

Treatment	Baseline Gingivitis Score	Final Gingivitis Score	Change
Chew A	1.12 \pm .17 ^a	1.05 \pm .34 ^a	- 0.07 \pm .23 ^a
Chew G	0.88 \pm .20 ^a	1.10 \pm .25 ^a	0.22 \pm .28 ^{ab}
Control (no chew)	0.92 \pm .24 ^a	1.72 \pm .23 ^b	0.80 \pm .20 ^b
P value	.51	.02	.03

Effect of treatment on dental plaque (mean whole mouth scores).

Treatment	Plaque Score	Std Error
Chew A	7.07 ^a	.97
Chew G	7.74 ^a	.65
Control (no chew)	11.42 ^b	1.19
P value	.0004	

Effect of treatment on dental calculus (whole mouth scores).

Treatment	Calculus Score	Std Error
Chew A	2.15 ^a	.40
Chew G	2.38 ^a	.41
Control (no chew)	4.20 ^b	.40
P value	.0001	

^{ab} Means within columns not sharing a common letter in the superscript differ significantly ($P < 0.05$)

morning during the remainder of the 2-week pre-test phase, using a double-ended toothbrush^f and dental paste^g. The purpose of the pre-test phase was to encourage clinically healthy gingivae prior to commencing the test phase. All dogs were maintained on the standard diet throughout the pre-test phase, but no dental chews were given.

On day 1 of the test phase, baseline gingivitis scores for each dog were recorded while the dogs were administered general anesthesia. The teeth were scaled and polished supragingivally to provide a clean tooth surface. There was no evidence of gingival recession or pockets when examined on day 1. Throughout the 4-week test phase, dogs in the control group were fed the standard diet only. All other dogs received one dental chew (A or G) each day. Dental chews were offered approximately 1-hour before feeding the standard diet. Any refusals of either dental chews or diets were recorded. On the last day of the 4-week test phase, dogs were anesthetized randomly and dental scoring procedures were performed to assess the levels of gingivitis, calculus, and plaque accumulation on the teeth of all dogs. Teeth were scaled and polished following dental scoring, in preparation for the next 2-week pre-test phase. For each dog, the following twenty-two teeth were scored for gingivitis, plaque, and calculus (Tables 1 - 3). Right and Left Maxilla: I3, C, P2, P3, P4, M1. Right and Left Mandible: C, P2, P3, P4, M1. Missing teeth were not scored, and mean values were calculated from the total number of teeth scored. Dental score data were initially screened for normality of data distribution using a statistics software package^h. No variables required transformation. Data were then subjected to analysis of variance appropriate to a Latin Square design using the super ANOVA program^h. The effects fitted in the model were Dog ($n = 12$), Period ($n = 3$) and Treatment ($n = 3$). No interactions were fitted as the experimental design does not allow detection of interaction between the main effects. Where the effect of treatment was significant ($P < 0.05$), the significance of

differences between individual treatment means was determined using Duncan's new Multiple Range test in the super ANOVA program. A value of $P < 0.05$ was considered significant.

Results

All dogs remained in good health throughout the study. Both the standard diet and the chews were highly palatable, and refusals were rare and inconsequential. Dogs fed a single daily dental chew (Chew A or Chew G) had significantly less gingivitis ($P = 0.02$), plaque ($P = 0.0004$), and calculus ($P = 0.0001$) than dogs in the Control group that were fed an identical diet but received no chews (Fig. 2 and Table 4). There was no significant difference between dogs fed Chew A or Chew G in any of the variables measured in this study. The inclusion of an anti-microbial agent in Chew G did not improve the efficacy of the product. Both products were equally effective at reducing gingivitis, plaque, and calculus in dogs.

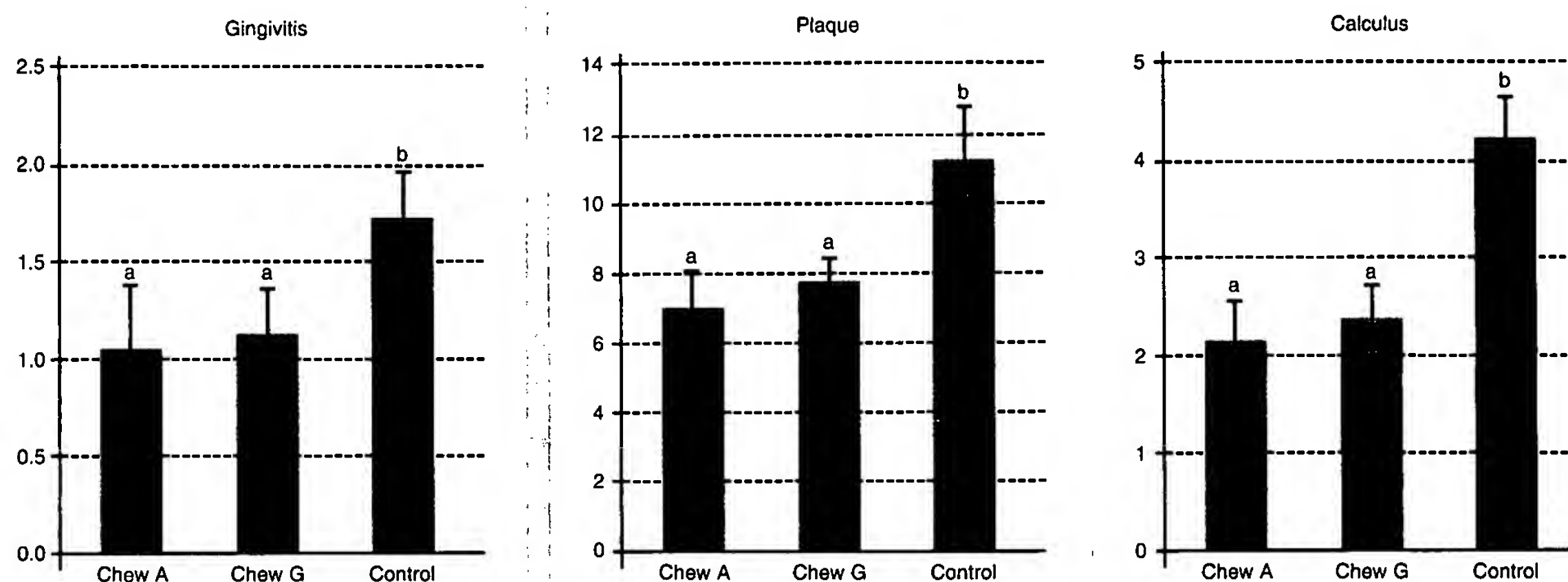
Discussion

In this study, the inclusion of a natural antimicrobial agent into the oral hygiene chew being tested had no effect on the efficacy of the product. In a similar study evaluating the effectiveness of dietary chews in dogs, the incorporation of an antimicrobial agent (chlorhexidine) resulted in significantly less plaque accumulation but made no difference to the levels of gingivitis or calculus²⁰. Highly significant results were obtained in this study with a product that did not have any known antimicrobial activity (Chew A), emphasising the importance of texture in products designed for improving dental health in dogs.

Standards set by the American Dental Association (ADA) require a statistically significant reduction of at least 15 % in gingivitis indices to claim that a product is clinically effective in controlling gingivitis²¹. In our study, we obtained a statistically significant reduction of 39 % with the daily inclusion of a dental chew (Chew A) to the standard diet. Similarly, the daily provision

Figure 2

Effect of treatments on the indices of periodontal disease (gingivitis, plaque, and calculus) in dogs. All dogs were fed an identical diet. Measurements were made after 4-weeks of feeding either the diet alone (Control), or the diet supplemented daily with Chew A (Pedigree® Denta Stix™) or Chew G (Pedigree® Denta Stix™ with an antimicrobial added). Data shown are group means (\pm SEM) of whole mouth scores for each dog ($n = 12$).



^{ab} Means within columns not sharing a common letter in the superscript differ significantly ($P < 0.05$)

of a single chew (Chew A) significantly reduced plaque and calculus by 38 % and 49 %, respectively.

The reference diet fed to the dogs in this study is representative of commercial diets commonly used by pet owners. The practice of feeding a dry diet combined with a canned food is reported to be the most popular dietary regimen used for pet dogs in both Australia and the UK. The results of this study, therefore, are arguably valid for the pet dog population at large. It would be reasonable to suggest that dog owners could expect similar improvements in the dental health of their dogs by feeding a daily dental chew of the type tested in this study.

- * Pedigree Denta Rask (Europe), and Pedigree Dentabone (North America)
- ^b Pedigree DentaStix, Masterfoods, Hungary
- ^c Pedigree Advance Adult – chicken, Uncle Ben's of Australia, Wodonga, Victoria
- ^d Pedigree Advance Energy – chicken and rice, Uncle Ben's of Australia, Wodonga, Victoria
- ^e Pedigree DentaStix, Masterfoods, Hungary
- ^f Dentipet, Arnolds, Shrewsbury, UK
- ^g Dentipet Premier dental paste for dogs and cats, Arnolds, Shrewsbury, UK
- ^h Staview II Abacus Concepts, Berkley, CA, USA
- ⁱ Red Cote 1.5% D&C Red No. 28, John O Butler Co, Chicago, IL, USA
- * Undisclosed, proprietary product

Author Information

From the University of New England Armidale, NSW 2351, Australia, and Masterfoods Complementary Petcare (McGenity), Oakwell Way, Birstall, Batley, West Yorkshire, WF 17 9LU, UK. Email: wbrown@une.edu.au

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